the literature, including other vibrational spectroscopic data and high-level simulations.

1701-Pos Board B611
Confocal Raman Spectroscopy of Collagen Hydration: from Human Type I Collagen to Dermal Tissue
Qihong Zhang, K.L. Andrew Chan, Guojin Zhang, Timothy Gillece, Laurence Senak, David J. Moore, Richard Mendelsohn, Carol R. Flach.
Collagen, representing approximately 75% of the dry weight of dermis, has a triple helical motif which consists of three polyproline II-like strands wrapped around a common axis. Studies of collagen's molecular and higher order structure have revealed the importance of water in stabilizing triple helical conformation and fiber assembly. A better understanding of collagen hydration is of interest to both medical and cosmetic industries. Confocal Raman spectroscopy, a noninvasive technique, has been used to measure the hydration properties of human Type I skin collagen and pigskin dermis at various relative humidity (RH) values. Raman spectra were also collected for Type I collagen and dermis samples upon exchange of a 100% RH H_2O to deuterium oxide (D_2O) environment. Changes observed in the Amide I and III spectral regions are consistent with modifications in hydrogen bonding as RH increased and H $\rightarrow$ D exchange took place. In particular, the relative intensity of the Raman peak at 938 cm^{-1}, thought to arise from a protein backbone C-C stretching mode, was identified as a marker sensitive to collagen-bound water. Dynamic vapor sorption (DVS) measurements were also carried out on intact dermis to provide a quantitative measure of dermal hydration. The DVS results for internally absorbed water are compared with the Raman results and previous reports using a variety of physical techniques.

1702-Pos Board B612
Optical Trapping Raman Spectroscopy of Protein and Membrane Interaction
Anjan Roy, Marcelo Nakaema, Timothy A. Keiderling.
To study the interdependence of external mechanical force on membrane structure we added the capability of mechanical force measurements into a single beam optical tweezers coupled to a Raman microscope. The optical trap serves as a handle to control the interaction of the membrane to an immobilized protein and it ensures the analyte is trapped in the focal volume of the laser and hence insures that we can collect spectra of single vesicle/ single cell systems. Force measurements allow us to correlate conformational changes to binding energetics.
An optical trap was constructed by incorporating a position sensitive detector (PSD) and a peizo translation stage to a home-built Raman microscope. The PSD allows for force measurements on the trapped species and trap stiffness. The peizo-stage facilitates $<$50 nm translation resolution. We tested this system on micron sized polystyrene beads. The bead was visually guided into the trap, while the power spectrum (force) was collected simultaneously with the Raman spectrum (structure). This was successfully repeated for E.coli in growth media. This simultaneous structure and force measurements were found to be feasible. To achieve our goal of correlated (not just simultaneous) studies we moved forward to vesicle systems.
Our Lab has previously studied structural changes in BLG binding to lipid vesicles. We further that by studying structural transitions in single Giant Unilamellar Vesicles (GUV) as it binds to BLG. BLG was immobilized on a glass surface and was mechanically driven towards an optically trapped vesicle. The backscattered Raman signal from the vesicle was recorded as a function of proximity of the vesicle to BLG and we correlated conformational changes in the vesicle with its binding to BLG. We present these findings of our new experimental tool.

1703-Pos Board B613
Ligand and Substrate Migration in Human Indoleamine 2,3-Dioxygenase
Karin Nienhaus, Elena Nickel, Changyuan Lu, Suyn-Ru Yeh, G. Ulrich Nienhaus.
Human indoleamine 2,3-dioxygenase (hIDO), a monomeric heme enzyme, catalyzes the oxidative degradation of L-tryptophan (L-Trp) and other indoleamine derivatives. Using Fourier transform infrared and optical absorption spectroscopy, we have investigated the interplay between ferrous hIDO, the ligand analog CO, and the physiological substrate L-Trp. These data provide the long-sought evidence for two distinct L-Trp binding sites. Upon photodissociation from the heme iron at T $>$ 200 K, CO escapes into the solvent. Concomitantly, L-Trp exits the active site and, depending on the L-Trp concentration, migrates to a secondary binding site or into the solvent. Although L-Trp is spectroscopically silent at this site, it is still noticeable due to its pronounced effect on the CO association kinetics, which are significantly slower than those of L-Trp-free hIDO. L-Trp returns to its initial site only after CO has rebound to the heme iron.

1704-Pos Board B614
DFT-Based Simulations of Ir Amide I' Spectra for a Small Protein in Solution
Johan Grahnen, **Jan Kubelka**.
Infrared (IR) amide I' spectra are widely used for investigations of the structural properties of proteins in aqueous solution. For analysis of the experimental data it is necessary to separate the spectral features due to the backbone conformation from those arising from other factors, in particular the interaction with solvent. We investigate the effects of solvation on amide I' spectra for a small 40-residue helix-turn-helix protein by theoretical simulations based on density functional theory (DFT). The vibrational force fields and intensity parameters for the protein amide backbone are constructed by transfer from smaller hepta-amide fragments; the side-chains are neglected in the DFT calculations. Solvent is modeled at two different levels: first as explicit water hydrogen bonded to the surface amide groups, treated at the same DFT level, and, second, using the electrostatic map approach combined with molecular dynamics (MD) simulation. Motional narrowing of the spectral bandshapes due to averaging over the fast solvent fluctuation is introduced by use of the time-averaging approximation (TAA). The simulations are compared with the experimental amide I', including two 13-C isotopically edited spectra, corrected for the side-chain signals. Both solvent models are consistent with the asymmetric experimental bandshape, which arises from the differential solvation of the amide backbone. However, the effects of 13-C isotopic labeling are best captured by the gas phase calculations.

1705-Pos Board B615
Effects of Solvent-Backbone Interactions on the Amide I Band of an Alanine-Rich Peptide
David Tran, Sean M. Decatur.
Solvent conditions have long been influential in protein folding to functional three-dimensional conformations. We can directly measure the degree of intra-peptide and solvent-peptide hydrogen bonds through the use of infrared spectroscopy. Examination of the temperature-dependence of the amide I' band can help elucidate the contributions of solvent-backbone interactions to the band position. Strong hydrogen bonding solvents like water shift the amide I bands to lower frequencies while weaker hydrogen bonding solvents, such as DMSO, shift the peaks to higher frequencies.
In this work, we investigated AY6 (Ac-AAKAAY-NH2), an unstructured peptide, in D2O, 40% trifluoroethanol:D2O, and DMSO to probe both solvent and amide group effects. The backbone atoms in AY6 exhibit marked desolvation in DMSO, as evidenced by shifting of the amide I' bands to higher frequencies compared to D2O. In addition, the slope of the plot of amide I' frequency versus temperature is very similar in each solvent. We compare these findings to a series of other model systems.

Imaging and Optical Microscopy II

1706-Pos Board B616
Orientation-Independent DIC Microscopy with Fast Switching Shear Direction and its Applications
Michael Shribak, Amitabh Verma.
A conventional DIC (differential interference contrast) microscope shows the two-dimensional distribution of refractive index gradient encountered along the shear direction. Thus contrast of DIC images varies proportionally with the cosine of the angle made by the azimuth of the refractive index gradient and the direction of wavefront shear. It is therefore necessary to examine unknown objects at several azimuth orientations.
To overcome the limitations of available systems, we have built an assembly, which allows the bias and shear directions to be switched rapidly without mechanically rotating the specimen or the prisms. The assembly consists of two standard Nomarski prisms with liquid crystal polarization rotator in between. When the polarization rotator is in OFF state, the total shear direction of the assembly is at +45-deg to the shear direction of the first prism. If the polarization rotator is in ON state, the total shear direction is at −45-deg to the shear direction of the first prism. Thus, when one would switch the polarization, the shear direction would be rotated by 90-deg.
We added one assembly to the illumination path and another one to the imaging path of the standard Olympus BX-61 microscope. Also one variable liquid crystal retarder was installed in order to change a bias. Four raw DIC images at two orthogonal shear directions and two inverse biases are captured and processed within a second. Then the quantitative image of refractive index distribution within a thin optical section is displayed on a computer screen. The orientation-independent DIC data obtained can also be used to compute the quantitative distribution of refractive index gradient or to generate enhanced, regular DIC images with any desired shear direction. The OI-DIC system